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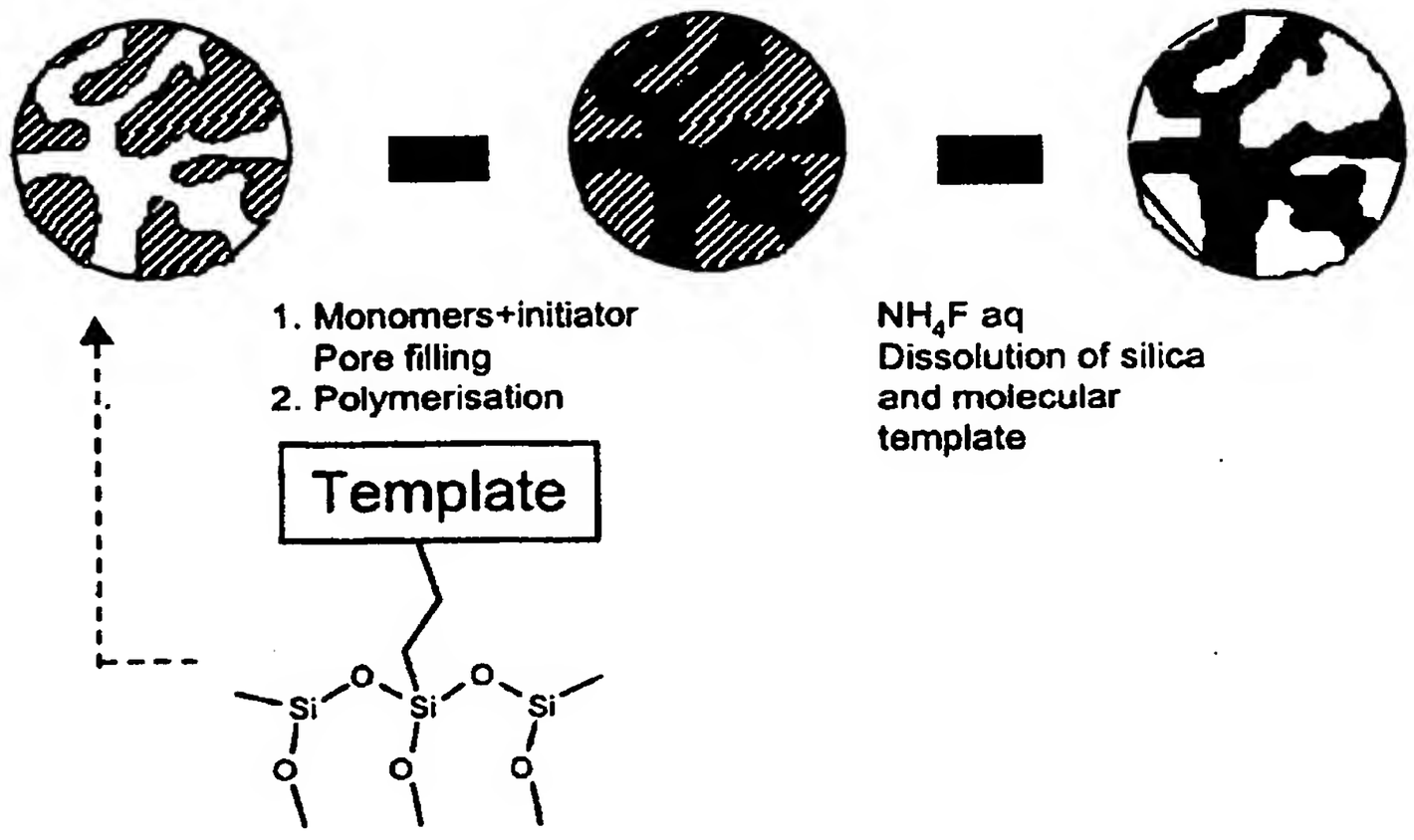
(54) Title: A POROUS, MOLECULARLY IMPRINTED POLYMER AND A PROCESS FOR THE PREPARATION THEREOF



(57) Abstract: A porous, molecularly imprinted polymer and a process for its preparation are described. The porous, molecularly imprinted polymer is characterised in that it is obtainable by providing a porous silica; attaching a molecular template to the surface of the porous silica; filling the pores of the porous silica with a polymer; removing the silica and the molecular template, thereby leaving a porous, molecularly imprinted polymer. The process is characterised by the above defined process steps. Also described are a porous polymer vesicle and its preparation with the same features as defined for the porous, molecularly imprinted polymer and its preparation, except for the lack of the molecular template and thus the lack of the molecular imprint in the porous polymer.

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A POROUS, MOLECULARLY IMPRINTED POLYMER AND A PROCESS FOR THE PREPARATION THEREOF

Field of the invention

Synthesis and use of porous network polymers and spherical polymer vesicles with molecular recognition properties by the use of mesoporous silica gel and core/shell silica gel particles as molecular pore- and cavity-templates.

The invention describes new nanostructured materials whith selective binding properties for small molecules and biological macromolecules.

Technical background

In molecular imprinting in the presence of a template, polymers (MIPs) with high level of cross-linking are produced. The template corresponds regarding its structure and functionality to the target molecule [1, 2, 3, 4] and is after the synthesis removed, whereby cavities with forms and functionalities according to the template, remain. A number of imprinted polymers have until now been produced and the imprinting process constitutes a very promising way regarding the accomplishment of a large number of selective separations. The MIPs are distinguished by their high selectivity and affinity. In this way numerous materials with antibodylike affinities can be produced. The advantages of the MIPs are their high association constants (K_a up to 10^8 M^{-1}) for the target molecule [5, 6] and their high stability. Disadvantages are the limited chromatographic separation capacity (broad and asymmetrical peaks at liquid-chromatography, LC), low load capacities (< 3mg/g), as well as a complicated preparation process with low yield. The low yield prevents a scaling up and the use of more expensive templates. Besides, there are only a few examples of successful imprinting of biological macromolecules.

(i) Production of polymers with molecular recognition properties (MIPs) as monodispersed particles.

Most of the MIPs are produced in the presence of a template through free radical polymerization of functional, unsaturated monomers (vinyl-, acryl- methacryl-) and an excess of di- or tri- unsaturated monomers (vinyl-, acryl-, methacryl-) as cross-linkers, where by porous, organic networks are produced. This method has the advantage that relatively stable polymers can be produced with the use of different solvents and at different temperatures [7], which is important in view of the different solubilities of the various templates.

Most of the non covalent molecular imprinting systems are based on acryl- or methacrylmonomers, as for instance methacrylic acid (MAA), which is cross-linked with ethyleneglycoldimethacrylate (EDMA). For the production of imprinted stationary phases for chiral separation (chiral stationary phases, CSPs) at the beginning derivates of amino acid enantiomers were used as templates. This system can generally be used for the imprinting of templates via hydrogen bonding or electrostatic interaction with MAA [8, 9]. The method is explicitly demonstrated by the example of L-Phenylalanin-anilid (L-PA).

In the first step the template (L-PA), the functional monomer (MAA) and the cross-linker (EDMA) are dissolved in a solvent with insignificant tendency towards forming hydrogen bonds and with small to average polarity. The free radical polymerization is thereafter started with an azo-initiator, for instance azo-N,N'-bis-isobutyronitril (AIBN), either photochemically at room temperature [11, 10] or thermochemically at 60°C or higher [11]. The MIPs are formed as monoliths and before they are used they have to be crushed with mortar and pestle or with a ball mill. Following sieving the particles are

sorted in the fractions 28-38 μm for chromatography resp. 150-200 μm for batch applications [11, 12, 13, 14, 15]. The template is extracted with a Soxhlet apparatus and thus recovered. The polymers as stationary phases are then evaluated by chromatography and the retention time and the capacity factors (k') [16] of the templates are compared with those of analog structures.

The work up of the polymers by crushing and sieving is associated with high costs and and a high loss of material in the form of fine particles. Besides it is difficult to produce these materials on a larger scale. By the sieving of the monolith particles irregular particles arise which not only have surface localized binding sites but also binding sites with poor accessibility. Due to flow disturbances and diffusion limitations this causes a poor separation performance in chromatography [17].

Therefore there is a need to produce MIP-materials in large quantities and with homogeneous morphology, as these regarding their mass transfer properies and their load capacity are superior compared to the irregular particles from the monolithmethod. Materials with homogenous morphology are already produced by bead polymerization [12, 18], dispersion polymerization [13] or precipitation polymerization [14]. The morphogy of these products are very sensitive to small changes regarding the synthesis conditions and besides, only certain solvents can be used for the polymerization. By consequence the synthesis for each target template has to be optimized which is costly and clearly limits the use of this synthesis variant. In addition, the synthesis conditions for the production of spherical particles are not always compatible with those synthesis conditions which lead to a higher selectivity and affinity for the template molecules. An alternative is the coating of preformed support materials [15, 19, 20], through which MIPs can be produced on metallic oxides. [15, 20] Another

is the coating of the MIPs on organic polymer supports [19] or on the walls of fused silica capillaries [21, 22, 23].

For instance, for the production of molecular imprinting polymer coatings, wide-pore silica gels (Silica 60, Silica 100, Silica 500 or Silica 1000 (Merck)), modified with 3-(Trimethoxysilyl)propyl-methacrylat and in connection treated with Hexamethyl-disilazan (end-capping) have been used. The support is then coated with a thin layer (10-156 Å) of a monomer mixture (ethylenglycoldimethacrylat, EGDMA and meth-acrylic acid, MAA) in the presence of a chiral template and an initiator (azo-bis(isobutyronitril), AIBN). After polymerization (monomer grafting approach) [15], the resulting silica gels were sieved by wind sieving and thereafter sedimented and tested chromatographically. An other method consists in the coating of LiChrosphere 1000 with a metal complexing polymer layer. This is performed by coating of propylmethacrylat derivatized silica particles with a metal complexing polymer in presence of a metal coordinating template [24].

(ii) Use of non porous silica particles as pore template and production of polymer vesicles

The production of mesoporous polymers by use of colloidal silica particles as template was described by Johnson et al. [25]. Silica gel particles between 15 and 35 nm were settled and stabilized under pressure and heat. The spaces in the thus produced agglomerates of silica gel particles were then filled with a mixture of divinylbenzene (DVB) and an initiator (AIBN) and the polymerization started by heating to 60°C. The silica template was then dissolved with hydrofluoric acid or ammonium resp. cesium fluoride, whereby a vesical polymer frame was obtained, where the pore diameter can be varied through the dimension of the collodial silica gel.

Micro- and nanovesicles, which allows the inclusion of different materials, can be produced by spontaneous self-assembly of for instance amphiphilic block-copolymers [26, 27] or phosphorous lipids [28], by emulsion polymerization or by coating of colloidal particles with organic multi-layered films. Thus it is possible to stepwise coat collodial melamin resin particles with polyelectrolyte molecules and subsequently dissolve the core [29]. The weakly cross linked melamine resin particles with density in the size range 0,1 to 10 μm serve as template, which can be dissolved at pH-values < 1,6. The template is repeatedly coated with alternately charged polyelectrolytes. This coating is also stable after having dissolved the template, whereby polyelectrolyte vesicles are obtained whose dimensions are determined by the dimension of the template. The strength of the wall is determined in advance by the coating and can within wide limits be freely determined. In such a way polyelectrolyte vesicles can be produced, which show a selective permeability for polymers depending on their molecular weight. [30].

The selective permeability can for instance be used in order to create ionic conditions inside the vesicles which differ significantly from the volume phase [31].

By the use of neutral templates of type $C_nH_{2n+1}NH\ (CH_2)_2NH_2$ and tetraethoxysilane as silica source mesoporous molecular sieve with vesical structure can be obtained [32]. The vesicle is formed with one or more ca. 3 - 70 nm thick, wavy silica layers. The silica layers have mesopores in the size range 2,7 - 4,0 nm. The silica vesicles which are in the size range ca. 20 nm to 1400 nm, show a high thermal and hydrothermal stability.

(iii) Production of non porous silica gel particles in the range of submicrometer and micrometer

The synthesis of non porous silica gel particles in the range of submicrometer and micrometer up to ca. 4 µm is based on works of Stöber et al. [33], whereby by hydrolysis and condensation of tetraalkoxysilanes in ammonia water and with ethanol as cosolvent monodisperse, spherical silica gel particles up to 1,6 µm, resp. polydisperse particles up to 3 µm are formed. The synthesis is exhaustively investigated in several reports [34, 35, 36]. The production of monodisperse silica gel particles up to 2,0 µm by hydrolysis and condensation of tetraethoxysilan at -20 °C [37] and the synthesis of spherical silica gel particles under acidic conditions have been described [38]. The synthesis of larger non porous silica gel particles up to 10 µm is performed in the two-phase system alkoxysilan/water, where, due to the ethanol formed during the reaction, the system is slowly transformed to one single phase. [39].

(iv) Synthesis of monodisperse, spherical, porous silica gel particles in the submicrometer and micrometer range

The production of porous silica gel particles in the range of micrometer (> 5 µm) is performed by emulsion polymerization [40]. These exhibit a wide particle size range and have to be sieved. Porous silica gel particles in the nanometer range (< 10 nm) have been described by Chu et al. [41]. The synthesis is based on a two-phase sol-gel process of silica without cosolvent.

Regarding the production of monodisperse, spherical, porous silica gel particles in the submicrometer range there is nothing known in the literature.

(v) Synthesis of monodisperse, spherical, mesoporous Core/Shell silica gel particles in the submicrometer range.

There is nothing known in the literature regarding synthesis of monodisperse, spherical silica gel particles with a non porous core and a mesoporous layer.

The present invention

The problem of poor site accessibility in molecularly imprinted polymers can in principle be solved through simultaneous use of porous and molecular templates as in this way a large number of molecular binding sites will be located on the surface. This procedure is called the double template method. In this invention we have developed a double template method which, through the use of porous silicagel, will make it possible to produce highly accessible molecularly imprinted binding sites in porous organic network polymers.

According to another aspect of the invention the use of core/shell particles with a porous shell and a nonporous core will make it possible to produce hollow vesicles with adjustable porosity. Combining the core/shell particles with the double template approach will allow the preparation of molecularly imprinted vesicles .

According to the present invention there is provided a porous, molecularly imprinted polymer, characterised in that it is obtainable by

providing a porous silica;

attaching a molecular template to the surface of the porous silica;

filling the pores of the porous silica with one or several monomers followed by polymerisation of the monomers;

removing the silica and the molecular template, thereby leaving behind a porous, molecularly imprinted polymer.

The present invention also provides a process for the preparation of a molecularly imprinted polymer, characterised by

providing a porous silica;

attaching a molecular template to the surface of the porous silica;

filling the pores of the porous silica with one or several monomers followed by polymerisation of the monomers;

removing the silica and the molecular template, thereby leaving behind a porous, molecularly imprinted polymer.

The present invention also provides a vesicle, characterised in that it is obtainable by

providing silica particles with a nonporous core and a porous shell (core/shell particles);

filling the pores of the core/shell particles with one or several monomers followed by polymerisation of the monomers;

removing the silica, thereby leaving behind a porous, polymer vesicle.

The present invention also provides a process for the preparation of a vesicle, characterised by

providing silica particles with a nonporous core and a porous shell (core/shell particles);

filling the pores of the core/shell particles with one or several monomers followed by polymerisation of the monomers;

removing the silica, thereby leaving behind a porous, polymer vesicle.

These and further characteristics and advantages of the present invention will become evident from the description given below and the apended claims.

Description of the drawings

Figure 1 schematically depicts a model system for the bonding of templates on the surface of silica gel and substrate for the test of the recognition of the corresponding templates;

Figure 2 schematically depicts the use of porous silica gel particles as pore template in molecular imprinting.

According to the the first aspect of the invention a new class of molecular imprinted polymers are to be produced, at which the polymer morphology is controlled through the use of porous silica and the molecular recognition will be controlled through surface bound templates. The porous silicas act as porous templates and the surface linked molecules as template in order to acquire binding sites for molecules on the silica surface. The template, linked on the surface, is surrounded by a coating with a monomer mixture followed by the polymerization of the latter. Two model systems are illustrated schematically in Figures 1 and 2.

In model system A nucleotide bases, oligonucleotides or derivatives thereof and in model system B aminoacid or peptide derivatives are bound to the surface through standard coupling chemistry. In Figure 1 the silica surface is first silanised with glycidoxypropyltrimethoxy silane (GPS) to produce the corresponding epoxymodified silica surface (SiOGPS). In model system A this surface is then reacted with a purine or pyrimidine base derivative such as 9-(2-aminoethyl)adenine). The pores of the silica particles are then filled with an appropriate monomer mixture, the monomers polymerised for instance by free radical polymerisation and then the silica template is dissolved out by treatment with fluoride, e.g. ammoniumfluoride. The binding sites resulting from the example in Figure 1 would be complementary to 9-ethyl-adenine. Instead of modified silica, the template can also be made of controlled pore glass (CPG) which allows the direct use of the synthesis products resulting from solid phase DNA synthesis to be used as templates. Thus oligonucleotidemodified CPG can be used to create materials with affinity for the same oligonuclueotides, or DNA or RNA containing corresponding sequences.

In model system B the SiOGPS surface is instead reacted with aminoacid or peptide derivatives. Here the N-protected peptide FMOC-Phe-Gly is reacted with the epoxygroups on the surface. After deprotection pore filling with monomers, polymerisation and silica-dissolution are carried out as described above and the rusulting material can then be used to selectively bind corresponding peptides or corresponding N-terminal epitopes.

The model systems thus allow the production of surface located binding sites for nucleotide bases, oligonucleotides, amino acids or peptides at which, owing to the dissolution of the silicagel, spongy, macroporous materials are formed which can recognize the corresponding template. As the binding sites are localized on the surface of meso- or macroporous materials their accessibility are especially high due to short diffusion paths. In this way it is possible to strongly bind biological macromolecules, as for instance oligonucleotides, polypeptides or proteins, which contain epitopes corresponding to the template. The process is schematically shown once again in figure 2.

This process can be transferred to other silica gel morphologies, in order to produce porous polymer vesicles. This is done through the polymerization of a monomer mixture in the pores of porous core/shell silicagel particles. These silicagel particles consist of a non porous core and a porous shell. The morphology and the porosity of the polymer vesicle are further adjusted through variations of the core/shell silicagel synthesis. To this end the core/shell synthesis will be combined with different methods to produce porous silicagels. By the use of alkylsilanes, ionized or neutral pore creators and by variations of the conditions for the synthesis (temperature, concentration) the pore diameter of the shell as well as the particle size can be adjusted. After monomer filling, polymerisation and dissolution of the

core/shell silica template, this will in turn allow vesicles with adjustable pore diameter to be prepared. Finally the synthesis of porous polymer vesicles can be combined with molecular imprinting.

The described methods make it possible to accomplish the double template method with a large number of different silica support materials of silica of different size and porosity. The use of porous silica supports as porous template combined with molecular imprinting for synthesis of surface imprinted particles with defined size and porosity is not known.

The research leading up to the present invention includes the following.

(i) MIPs with new morphologies

(a) Monolith process

A model system for the production of MIPs according to the monolith process has been developed. To this end derivatives of amino acid enantiomers, for instance (e.g. L-phenylalanineanilid (L-PA) and the nucleotidebase derivative(9-Ethyladenin) were used as template. These interact via hydrogen bonding and electrostatic interactions with the functional monomer (MAA). For the photochemical polymerization at room temperature AIBN was used as initiator. After crushing and sieving of the polymers and after extraction of the template the material was evaluated in chromathography regarding its selectivity for the template. It functions as model system with which materials obtained according to the double template principle have to be be compared.

(b) Polymer coating of spherical silica supports

For the production of spherical molecularly imprinted particles non porous, spherical silica gel

(particle diameter: 1-5 μm) and porous, spherical silica gel (particle diameter: 10 μm, pore diameter: 1000 Å) are used. These were coated with the MIPs according to two different methods of which one, according to Wulff et al., consisted of modification of the surface with 3-(trimethoxysily)propylmethacrylate followed by reaction of remaining silanol groups (endcapping). After that the silica gel was coated with the monomer mixture (MAA, EDMA) containing the template (L-PA) and the initiator (AIBN) the polymerization was photochemically started.

A further way to coat the silica gels with MIPs is possible by a technique based on a method described by Guyot et al.. In this case the silica surface was first silanized with 3-aminopropyltriethoxysilane or glycidoxypropylsilane (GPS). Subsequently the amino resp. epoxy group were reacted with the initiator (Azo-bis(cyanopentanoic acid, ACPA). After coating with the monomer mixture the polymerization was photochemically carried out where the particles were suspended in the monomer mixture.

(ii) Synthesis of porous, spherical silica gel particles using different pore creators

(a) Alkyltrialkoxysilane (Octadecyltrimethoxysilane, C18-TMS) as pore creator

By cohydrolysis and cocondensation of tetraethoxy-silane (TEOS) and octadecyltrimethoxysilane as reactive silane in ammonia water and with ethanol as cosolvent spherical organo silica gel composites are created. These composites can be transformed into mesoporous, spherical silica gel particles by calcination at 550 °C. The material shows a specific surface a_s (BET) up to 750 m^2/g, a specific pore volume (Gurwitsch) up to 0.6 mL/g and an average pore diameter, according to synthesis conditions, between 2 nm and 4 nm. By variation of the reaction

temperature and the concentration of water resp. ammonia the particle size can be regulated up to 900 nm [42, 43, 44, 45].

(b) Neutral templates (alkylamines) as pore creators

The use of n-alkylamines for the synthesis of mesoporous metal oxides was first tried by T.J. Pinnavaia [46]. These products showed an irregular morphology. By changing to a homogenous reaction system through the use of alcohol as cosolvent, the use of ammonia as catalyst and by variation of the concentration of the template and the silica-precursor it has been possible to obtain mesopouros and spherical SMS-materials (spherical mesoporous amine-templated silica) [47]. These particles have a diameter of up to 2 μm and show a specific surface, a_s (BET), up to 800 m^2/g, a specific pore volume (Gurwitsch) up to 0,8 mL/g and an average pore diamater (Wheeler) of 3,0.

(c) Ionic tensides (n-Alkyltrimethylammoniumbromide) as template

The synthesis of mesoporous metal oxides of the M41S class and their use as cracking catalyst for the petrochemical application dates back to works by scientist at the Mobil Oil Corporation from the year 1992 and back [48, 49]. This is examplified by the highly porous MCM-41 with its hexagonal arranged, cylindrical pore system. Through the synthesis of these materials in a single-phase system, i.e. through the use of tetraethoxysilane as silica source, alcohol as cosolvent and aqueous ammonia as catalyst, it has been possible to produce spherical particles with MCM-41-analog structure [48, 49, 50]. These materials have, each according to length of alkylchain and concentration of the templates a specific surface, a_s (BET), up to 1300 m^2/g, a specific

pore volume (Gurwitsch) up to 0,8 mL/g and a pore diameter (Wheeler) of ca. 2,0 nm.

(d) Polymers (Polyethylenoxide) as pore creators

For synthesis of spherical silica gel particles with pore diameter up to 50 nm polyethylenoxide (PEO) dissolved in a mixture of water, alcohol and ammonia is used. By adding TEOS, ca. 200 nm large, spherical silica gel composites are produced, which after filtration, drying and calcination have got a specific surface a_s (BET) up to 550 m^2/g and a specific pore volume up to 0,9 mL/g [51].

(iii) Synthesis of mesoporous core/shell silica gel particles

The synthesis of mesoporous core/shell silica gel particles depends on the combination of three known methods: at first the non porous silica gel core is produced according to the well-known Stöber-method [33-36]. The size can be adjusted between 100 nm and 3,0 μm by variations of the reaction temperature, the concentration of the water and the ammonia and by variation of the kind and concentration of the alkoxysilane and the alcohol. The following synthesis of the porous layer is based upon a combination of two well-known synthesis methods: on the one hand the synthesis of porous silica gel particles through cohydrolysis and cocondensation of tetraethoxysilane and C18-TMS [42-45], the method of the post growth on the other hand was originally developped for the enlargement of non porous silica gel particles [52]. In this way it was possible by adding tetraethoxysilane drop by drop to a suspension of spherical, non porous silica gel particles in water, ethanol and ammonia, to increase the particle size from

originally 500 nm to 3 μm. For the synthesis of the core/shell silica gel particles, a mixture of TEOS/C18-TMS, without any further processing, is dropped directly into the reaction suspension of the non porous Stöberparticles. The porosity is obtained by a following calcination at 550°C [45, [53], [54]].

By varying the quantity of added pore creator it is possible to produce mesoporous core/shell silica gel particles with a specific surface a_s (BET) of up to 350 m^2/g, a specific pore volume v_p (Gurwitsch) up to 0,4 ml/g and an average pore diameter p_d (BJH, des.) of 3,8 nm.

Particular embodiments of the invention

(i) Accomplishment of the double template principle using porous silica supports

The techniques used to coat polymers on non porous or macroporous silica gels shall be extended to pore filling of porous silica supports. Thereby serves a porous silica gel as morphological template and a surface bound template molecule as molecular template.

Porous silica will be used as spherical silica gels. Pore systems of different size and structure are then available through the different techniques to prepare porous spherical silica gels. Then the molecular template, as described in Fig. 1, is bound to the silica surface. Two model systems are available, at which in the first a peptide and in the second a nucleotide are fixed on the surface. Then the pore system of the porous, template modified silica gel is, evacuated and filled with a monomer mixture followed by a polymerization, according to the process of Mallouk.[25]

Thereafter the silica gel is dissolved using hydrofluoric acid or calcium or ammonium fluoride, whereby a porous, spherical polymer with binding sites localized on the surface is obtained.

The polymerization of the monomer mixture can take place in the pores of mesoporous, template-modified silica gels (Fig. 1 and 2). Hereby serves a number of different porous silica gel materials as support materials. For example spherical silica gels with a cylindrical pore system (M41S Class) can be used herefor, silica gels, which are composed of a nanoparticle agglomerate (SMS-Class resp. n-Alkyltrialkoxysilane as pore creators) or silica gels with polymers as pore creators. In this way different pore systems with regard to their size and structure are available. The silica surface is then to be silanisized with for instance glycidoxypropylsilane (GPS) and in connection reacted with the aminogroup of the templates (9-(2-amino-ethyl)adenine), model system I) or the carboxyl group of the templates (FMOC-L-Phe-L-Gly, model system II).

Instead of modified silica, the template can also be made of controlled pore glass (CPG) which allows the direct use of the synthesis products resulting from solid phase DNA synthesis to be used as templates. Thus oligonucleotidemodified CPG can be used to create materials with affinity for the same oligonuclueotides, or DNA or RNA containing corresponding sequences.

After removing of the solvent and evacuation of the silica or CPG pore system this is filled with the monomer mixture (MAA, EDMA) and the polymerization is photochemically started using AIBN. Then the silica gel is dissolved out with hydrofluoric acid or calcium or ammonium fluoride, by which a porous, spherical spongy polymer with affinity to the target molecule is obtained.

(ii) Production of micro- and nanovesicles using mesoporous core/shell silica gel particles

The under (i) described method for porefilling of porous silica gels can be used for filling the pores of porous core/shell silica gels, i.e. silica gels with a

non porous core and a porous shell. After polymerisation of the monomer mixture and dissolving the silica core with hydrofluoric acid or fluorides a porous polymer vesicle remains.

The size and porosity of the mesoporous core/shell silica gel particles can be further adjusted to requirements for chromatographic materials, whereby a particle diameter of over 2 μm is the objective. This is made by adjustment of the synthesis conditions for the core/shell silica gel particle. Through different methods regarding the synthesis of porous, spherical silica gel particles, support materials of different size, pore diameter and pore symmetri are available. By the thickness of the porous layer the specific surface area and the stability of the thus resulting polymer vesicle can then also be increased.

The process for coating of porous silica gels described in step (i) can be transferred to the coating of porous core/shell materials. Thus the template (L-Phe-L-Gly or a nucleotide base) can directly react with the GPS-silanisized silica surface (Fig. 1). After that the pore system is filled with the monomer mixture (MAA, EDMA) and the polymerization is performed with AIBN as initiator. By dissolving out the silica core, for instance with hydrofluoric acid or ammonium fluoride, porous polymer vesicles are formed, the size and porosity of which is decided through the porous properties of the corresponding core/shell particle. The mechanical stability can be by increasing the polymer thickness, i.e. as a result of the size of the porous layer of the core/shell particle and the pore diameter can be adjusted through the pore diameter of the core/shell particle. By drop by drop addition of TEOS/C18-TMS mixture and by dilution of the silica gel suspension during the after growth process, agglomeration can be prevented also at larger quantities of added silane mixture [55]. The porous core/shell silica gel particles can be be adapted

regarding their size and porosity according to the requirements on the resulting micro vesicles. Hereby the aim is to obtain spherical particles with a diameter of more than 2 μm, as otherwise in the HPLC, the pressure in the chromatographic column will be too high. This can be obtained by enlargement of the non porous silica core. The core particle size can be raised by variation of the reaction temperature, the solvent, the ammonia concentration and the alkoxysilane source. In this way the non porous silica core can be enlarged by up to 3,0 μm. References to this are to be found in the literature. Further on spherical silica gel particles up to 10μm are obtainable by synthesis in two-phase systems [41].

By combination with the already known process for the production of mesoporous silica gel particles, the porosity can be varied within the total mesoporous area, i.e. between 2 and 50 nm. For this purpose ionic (n-alkyltrimetylamminiumbromide) and non ionic template (n-alkylamine) and polymers (polyethylenoxide) are suited. These processes are all based on the hydrolysis and condensation of tetraalkoxysilanes in the presence of a pore creator in ethanol solution with ammonia as catalyst. It can then be combined with the already known methods for synthesis of core/shell silica gel particles.

The use of the porous core/shell silica gel particles provides the advantage of the flexible adjustment of specific surface and pore diameter. In this way the size of the polymer vesicle and the porosity and thickness of the vesicle wall can be carefully tuned.

(iii) Production of porous micro- and nanovesicles with molecular recognition properties

The process can be extended to vesicles with molecular recognition properties. For this purpose, a template (see Fig. 1) is required, which is bound to the particle surface or added as a mixture with the monomer.

After the following polymerization and after the extraction of the template the core will be dissolved and thus a porous polymer vesicle will be formed, exhibiting a pronounced affinity for the template.

To this end either the template 9-(2-aminoethyl)-adenine, (model system I) or FMOC-L-Phe-L-Gly, (model system II) can be used. These react with the epoxygroups of the silica surface bound GPS. After processing of the products the pores are filled with the monomer mixture and the initiator (AIBN) and polymerized. After the following polymerization the template and the silica core are dissolved leaving behind a spherical, porous polymer vesicle which shows a high affinity to the target molecule.

(iv) The use of spherical molecularly imprinted particles and porous core/shell silica gel particles

The spherical MIPs and the spherical polymer vesicles can be compared chromatographically regarding their selectivity and load capacity with the through the monolithprocess produced reference material. For this purpose, liquid chromatography (LC) or high performance liquid chromatography (HPLC) will be used according to stability of the materials. The porous polymer vesicles can further be used for storing, protection and target oriented liberation of drugs (Slow Release System).

Beside the use in chromatography, the porous core/shell silica gel particles may, after entrapment of metals, constitute potential support materials for catalytic applications.

The obtained porous, spherical polymers and the porous polymer vesicles are tested chromatographically (LC bzw. HPLC) regarding their selectivity and compared with the materials from the monolith process.

For the porous polymer vesicles there are, beside chromatography, a wide field of applications. Vesicles

can be prepared by controlled coating with several polymer layers on the silica surface. Such vesicles can for instance on the inside wall particularly contain amino groups, and on the outside wall on the contrary carboxyl or sulphonate groups. Furthermore, vesicles with amphiphilic groups in the interior of the capsules and hydrophilic groups on the outer wall can also be produced. By successive exchange of the aqueous solvent interior of the vesicles with an oil stable oil/water emulsions can be produced.

The porous polymer vesicles can further be used for storing and target oriented liberation of drugs (Slow Release System). The vesicle membrane exhibits due to its porosity and its chemical structure, a selective permeability which can be adjusted depending on planned application. The selective permeability of the vesicle walls can be used for instance via the setting of a Donann-partitioning in the interior of the capsules resulting in ionic conditions, which are different from the volume phase. Thus can for instance, due to different pH values in the interior of the vesicles and in the volume phase, selective chemical reactions inside the vesicle be carried out.

The porous core/shell silica gel particles have a high specific surface, which is concentrated within a thin shell on the surface of spherical silica gels. By silanization, reverse phase silica gel materials can be produced, which because of faster mass transport inside the silica gel particle should show distinct improvments compared to existing chromatographic stationary phases.

Besides the use in the chromatography the porous core/shell silica gel particles can, after doping with metals (Ni, Mo, Pt, etc.), function as potential support materials for catalytic applications. In addition the core/shell silica gel particles show a higher thermal and hydrothermal stability than common mesoporous silica gel

materials [48], which is important for catalytic applications.

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CLAIMS

1. A porous, molecularly imprinted polymer, c h a r a c t e r i s e d in that it is obtainable by

providing a porous silica;

attaching a molecular template to the surface of the porous silica;

filling the pores of the porous silica with one or several monomers followed by polymerisation of the monomers;

removing the silica and the molecular template, thereby leaving behind a porous, molecularly imprinted polymer.

2. A porous, molecularly imprinted polymer according to claim 1, wherein the polymer is comprised of porous particles.

3. A porous, molecularly imprinted polymer according to claim 2, wherein the particles are spherical particles.

4. A porous, molecularly imprinted polymer according to any one of claims 2 or 3, wherein the polymer is comprised of vesicular particles.

5. A porous, molecularly imprinted polymer according to any one of claims 2-4, wherein the particles have an average particle size of at least 2 μm.

6. A porous, molecularly imprinted polymer according to claim 5, wherein the particles have an average particle size of 2-10 μm.

7. A process for the preparation of a molecularly imprinted polymer, c h a r a c t e r i s e d by

providing a porous silica;

attaching a molecular template to the surface of the porous silica;

filling the pores of the porous silica with one or several monomers followed by polymerisation of the monomers;

removing the silica and the molecular template, thereby leaving behind a porous, molecularly imprinted polymer.

8. A process according to claim 7, wherein the porous silica comprises particles.

9. A process according to claim 8, wherein the particles are spherical particles.

10. A process according to claim 8 or 9, wherein the particles are of a core/shell type having a non-porous core of silica surrounded by porous silica.

11. A process according to any one of claims 7-10, wherein the porous silica is mesoporous, i.e. it has a pore size of 2-50 nm.

12. A process according to any one of claims 7-11, wherein the porous silica particles have a particle size of at least 2 µm.

13. A process according to any one of claims 7-12, wherein the molecular template is covalently attached to the surface of the porous silica.

14. A process according to any one of claims 7-13, wherein the pores of the porous silica are filled with the polymer by introducing at least one monomer into the pores and polymerising it into a polymer.

15. A process according to any one of claims 7-14, wherein the porous silica and the molecular template are removed by dissolution with a solvent.

16. A process according to claim 15, wherein the solvent is selected from hydrofluoric acid, calcium fluoride, and ammonium fluoride.

17. A porous polymer vesicle, c h a c t e r i s e d in that it is obtainable by

providing silica particles with a nonporous core and a porous shell (core/shell particles);

filling the pores of the core/shell particles with one or several monomers followed by polymerisation of the monomers;

removing the silica, thereby leaving behind a porous, polymer vesicle.

18. A process for the preparation of a porous polymer vesicle, characterised by

providing silica particles with a nonporous core and a porous shell (core/shell particles);

filling the pores of the core/shell particles with one or several monomers followed by polymerisation of the monomers;

removing the silica, thereby leaving behind a porous, polymer vesicle.

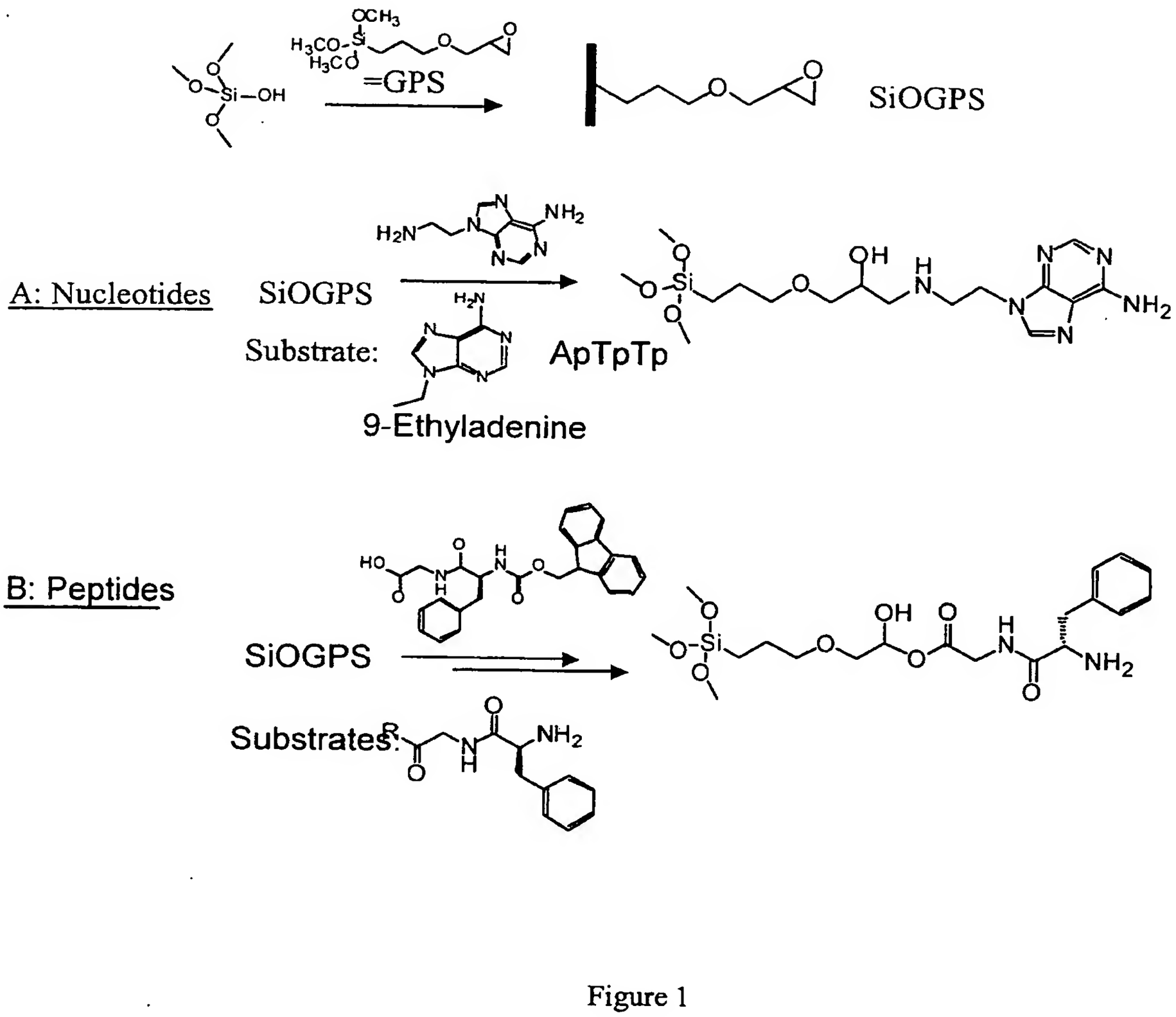
=GPS
SiOGPS
A: Nucleotides
SiOGPS
Substrate:
ApTpTp
9-Ethyladenine
B: Peptides
SiOGPS
Substrates:

Figure 1

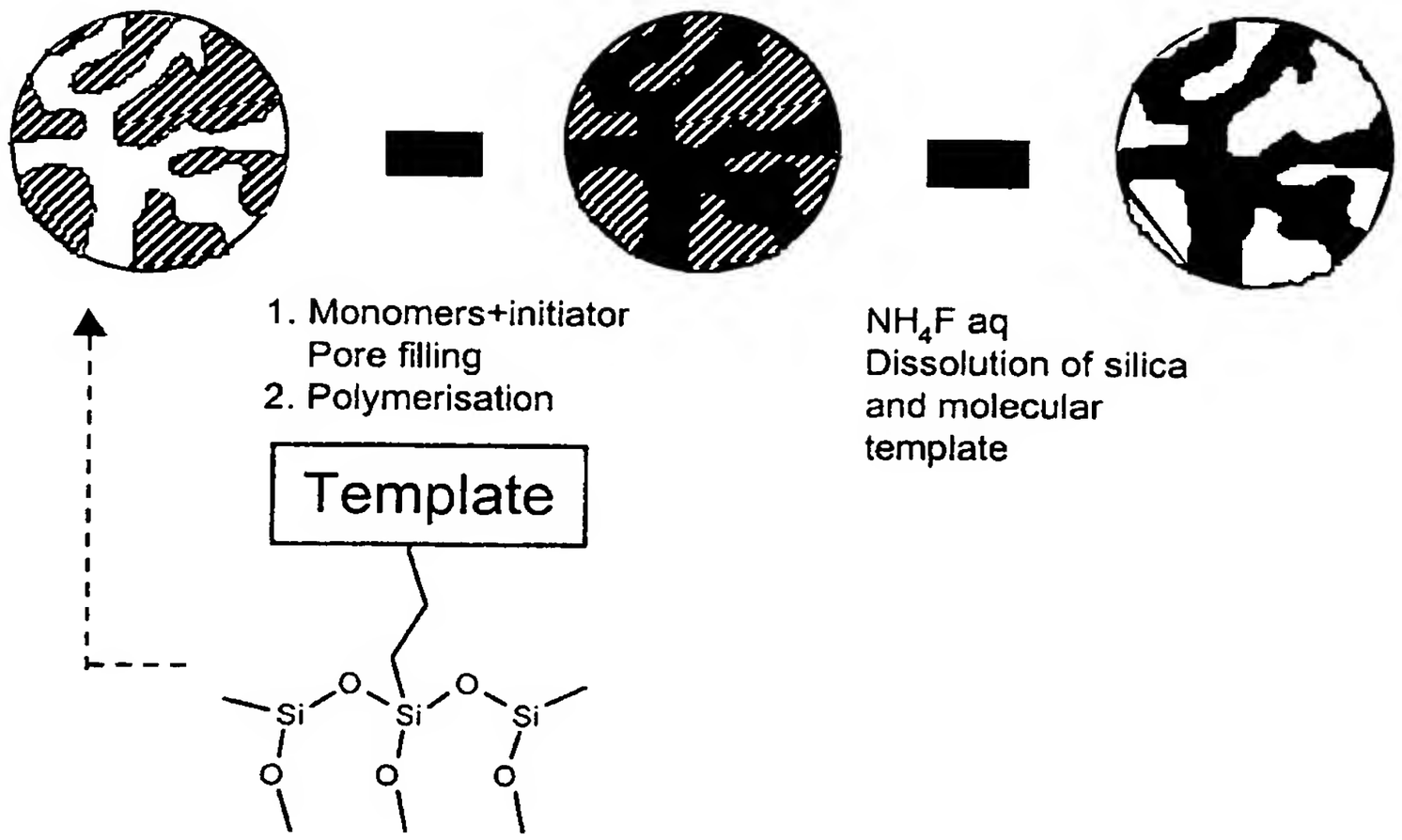
1. Monomers+initiator
Pore filling
2. Polymerisation
Template
Si
O
NH_4F aq
Dissolution of silica
and molecular
template

Figure 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 00/02083

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C08J 9/26 // G01N 33/53, G01N 30/48, C07K 1/22, C07K 17/08, A61K 9/52
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C08J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DERWENT/WPI, STN/CAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	Angew. Chem. Int. Ed., Volume 39, No 12, 2000, Yilmaz E. et al, "The Use of Immobilized Templates - A New Approach in Molecular Imprinting" page 2115 - page 2118 --	1-3,5-9, 12-16
X	WO 9521673 A1 (MOSBACH, KLAUS), 17 August 1995 (17.08.95), figure 5, example 3, claims, abstract --	1-3,5-9, 12-16
A	Reactive Polymers, Volume 3, 1985, Günter Wulff et al, "Enzyme-Analogue Built Polymers, 18* Chiral Cavities in Polymer Layers Coated on Wide-Pore Silica", page 261 - page 275, abstract --	1-3,5-9, 12-16

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 February 2001	23-02-2001
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Monika Bohlin/ELY Telephone No. +46 8 782 25 00

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 00/02083

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Journal of Chromatography, Volume 299, 1984, Olof Norrlöw et al, "Acrylic Polymer Preparations Containing Recognition sites obtained by imprinting with substrates", page 29 - page 41, abstract --	1-3,5-9, 12-16
A	Reactive Polymers, Volume 25, 1995, Glad M et al, "Molecularly imprinted composite polymers based on trimethylolpropane trimethacrylate (TRIM) particles for efficient enantiomeric separations", page 47 - page 54, abstract --	1-3,5-9, 12-16
A	Journal of Chromatography, Volume 347, 1985, Glad M et al, "Use of Silane Monomers for Molecular Imprinting and Enzyme Entrapment in Polysiloxane-Coated Porous Silica", page 11 - page 23, see page 12, last paragraph, lines 41-42 --	1-3,5-9, 12-16
A	US 5786428 A (ARNOLD ET AL), 28 July 1998 (28.07.98), see page 16, second and fourth paragraphs --	1-3,5-9, 12-16
A	WO 9637527 A1 (IGEN, INC.), 28 November 1996 (28.11.96), page 4, line 13 - line 19, abstract --	1-3
A	Angew. Chem. Int. Ed., Volume 38, No 9, 1999, Sheng Dai et al, "Imprinting Coating: A Novel Synthesis of Selective Functionalized Ordered Mesoporous Sorbents" page 1235 - page 1239 -- --------	1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE00/02083

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. A porous, molecularly imprinted polymer and a process for its preparation according to claims 1-16.

2. A porous polymer vesicle and a process for its preparation according to claims 17-18.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/SE 00/02083

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9521673 A1	17/08/95	AT 189130 T	15/02/00
		AU 1828095 A	29/08/95
		AU 6901794 A	20/12/94
		DE 69423487 D	00/00/00
		DE 69514785 D,T	31/08/00
		DK 743870 T	24/07/00
		EP 0700246 A,B	13/03/96
		EP 0743870 A,B	27/11/96
		SE 0743870 T3	
		EP 0982591 A	01/03/00
		JP 9510699 T	28/10/97
		NO 180512 B,C	27/01/97
		NO 954772 A	22/01/96
		SE 9400450 D	00/00/00
		US 5715773 A	10/02/98
		US 6127154 A	03/10/00
US 5786428 A	28/07/98	NONE	
WO 9637527 A1	28/11/96	AU 5859796 A	11/12/96
		EP 0828767 A	18/03/98
		JP 11507402 T	29/06/99
		US 5821311 A	13/10/98
		US 5872198 A	16/02/99
		US 5959050 A	28/09/99